

# On unbiased performance evaluation for protein inference

Zengyou He <sup>\*</sup>, Ting Huang and Peijun Zhu

School of Software, Dalian University of Technology, Dalian 116620, China.

Received on XXXXX; revised on XXXXX; accepted on XXXXX

Associate Editor: XXXXXXXX

Contact: zyhe@dlut.edu.cn

## 1 BACKGROUND

This letter is a response to the comments of Serang (2012) on Huang and He (2012) in *Bioinformatics*. Serang (2012) claimed that the parameters for the Fido algorithm should be specified using the grid search method in Serang *et al.* (2010) so as to generate a deserved accuracy in performance comparison. It seems that it is an argument on parameter tuning. However, it is indeed the issue of how to conduct an unbiased performance evaluation for comparing different protein inference algorithms. In this letter, we would explain why don't we use the grid search for parameter selection in Huang and He (2012) and show that this procedure may result in an over-estimated performance that is unfair to competing algorithms. In fact, this issue has also been pointed out by Li and Radivojac (2012).

## 2 MODEL SELECTION AND ASSESSMENT IN PROTEIN INFERENCE

Machine learning is a cornerstone of modern bioinformatics. Meanwhile, an unbiased performance evaluation is undoubtedly the cornerstone of machine learning research and applications (Cawley and Talbot, 2010), which provides a clear picture of the strengths and weaknesses of existing approaches.

In the real world application of machine learning methods, there are two closely related and separate problems: model selection and model assessment (Hastie *et al.*, 2009). In model selection, we estimate the performance of different models in order to choose the best one. In model assessment or performance evaluation, we test the prediction error of a final model obtained from the model selection process.

The protein inference problem is an instance of prediction task in machine learning as well, as shown in Fig.1. In model selection, we use the peptide-protein bipartite graph as the input to find a “best” inference model that produces a vector  $\hat{Y}$  (Huang *et al.*, 2012). Each element in  $\hat{Y}$  can be either the probability/score that each protein is present or the presence status of each protein (true or false). In model assessment, we compare the predicted vector  $\hat{Y}$  with ground-truth vector  $Y$  to obtain the performance estimates. This is the correct procedure for evaluating and comparing protein inference algorithms.

<sup>\*</sup>to whom correspondence should be addressed

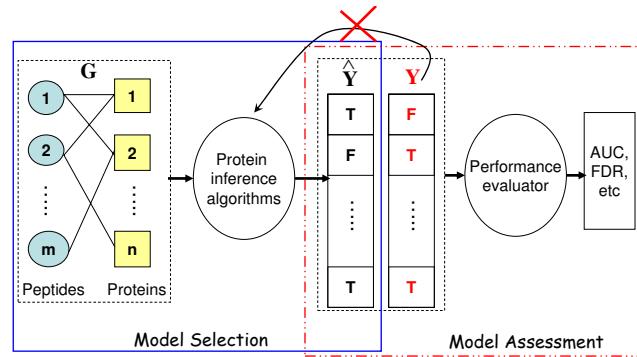


Fig. 1: The correct and incorrect procedure for assessing the performance of protein inference algorithms. In model selection, we cannot use any ground-truth information that should be only visible in the model assessment stage. Otherwise, we may over-estimate the actual performance of inference algorithms.

In contrast, one possible mistake in an incorrect procedure is illustrated at the top of Fig.1: the partial or whole ground-truth vector  $Y$  is used in the model selection process of the protein inference algorithms. The problem is that the inference algorithms have an unfair advantage since they “have already seen” the absence/presence information in  $Y$  that should only be available during model assessment. In other words, the ground-truth information has leaked to the model selection phase. As a result, the performance estimates of inference algorithms will be over optimistic. This phenomenon is essentially analogous to the selection bias observed in classification or regression due to feature selection over all samples prior to performance evaluation (Smialowski *et al.*, 2010).

According to the description in Serang *et al.* (2010) and the source codes of Fido, the grid search procedure chooses the set of parameters that jointly maximizes the  $ROC_{50}$  score (the average sensitivity when allowing between zero and 50 false positives) and minimizes the mean squared error (MSE) from an ideally calibrated probability. Clearly, it has used the ground-truth information (true and false positive labels)<sup>1</sup> that should only be available in the model

<sup>1</sup> In the target-decoy database search and evaluation strategy, a protein is regarded as a true positive if it comes from the target database and as a false positive otherwise. Therefore, the set of target/decoy labels is used as the set of ground-truth labels in this context, although some target proteins may be false positives.

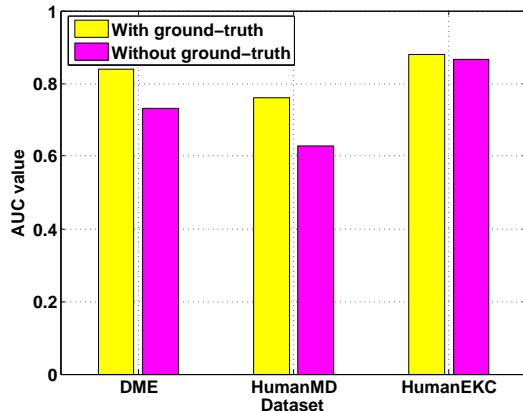


Fig. 2: The effect of using the ground-truth information in the grid search procedure of Fido. The grid search procedure finds a set of parameters automatically with the ground-truth labels of candidate proteins as input. Note that such presence/absence information of proteins should not be visible to the inference algorithms if they are once again used in the performance evaluation stage for comparing different algorithms. To mimic the situation that the ground-truth information is unavailable, we assign a zero weight to  $ROC_{50}$  in the grid search method and calculate the average area under curve (AUC) value as the performance index of “without ground-truth”.

assessment stage. In particular, the grid search procedure selects parameters using the  $ROC_{50}$  score as a key factor, which is directly related to the final performance index in the model assessment stage. Therefore, it is highly possible that over-fitting occurs, i.e., the use of grid search will lead to a performance overestimation.

To check if the grid search method will lead to an over-optimistic performance, we conduct the following experiment. As this procedure can control for how much weight should be given to  $ROC_{50}$  and how much weight should be given to MSE in model selection, we first assign a zero weight to  $ROC_{50}$  to roughly mimic the situation that the ground-truth information is invisible so that no over-estimation occurs. Then, we compare its performance with that given by the algorithm when the default non-zero weight is used for  $ROC_{50}$ . As shown in Fig.2, the performance of Fido will be deceased when the ground-truth information (in terms of  $ROC_{50}$ ) is not used in model selection. One may argue that we cannot fully

attribute the performance gain in grid search to the incorrect use of ground-truth information, but at least, it will be unfair to other competing algorithms in performance comparison.

### 3 SUMMARY

The fact that over-fitting at the level of model selection can have a very substantial deleterious effect in performance evaluation has been widely discussed and recognized in machine learning research (Cawley and Talbot, 2010) and bioinformatics society (Smialowski *et al.*, 2010). In protein inference, we will face the same problem as well. The main objective of this letter is to highlight this fact and people should be aware of such risk in future comparison when developing new protein inference algorithms.

*Funding:* The Natural Science Foundation of China under Grant No.61003176 and 61073051.

*Conflict of Interest:* None declared.

### REFERENCES

Cawley,G. and Talbot,N. (2010) On over-fitting in model selection and subsequent selection bias in performance evaluation. *Journal of Machine Learning Research*, **11**, 2079–2107.

Hastie,T., Tibshirani,R. and Friedman,J. (2009) *The elements of statistical learning: data mining, inference, and prediction*. Springer.

Huang,T. and He,Z. (2012) A linear programming model for protein inference problem in shotgun proteomics. *Bioinformatics*, **28**, doi:10.1093/bioinformatics/bts540.

Huang,T., Wang,J., Yu,W. and He,Z. (2012) Protein inference: a review. *Briefings in Bioinformatics*, **13** (5), 586–614.

Li,Y.F. and Radivojac,P. (2012) Computational approaches to protein inference in shotgun proteomics. *BMC Bioinformatics*, **13** (Suppl 16), S4.

Serang,O. (2012) Concerning the accuracy of fido and parameter choice. *Bioinformatics*, doi:10.1093/bioinformatics/bts687.

Serang,O., MacCoss,M.J. and Noble,W.S. (2010) Efficient marginalization to compute protein posterior probabilities from shotgun mass spectrometry data. *Journal of Proteome Research*, **9** (10), 5346–5357.

Smialowski,P., Frishman,D. and Kramer,S. (2010) Pitfalls of supervised feature selection. *Bioinformatics*, **26** (3), 440–443.